Minimizing fear and anxiety in working dogs: A review

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Abstract

The causes of fear and anxiety in working dogs are multifactorial and may include inherited characteristics that differ between individuals (e.g. Goddard and Beilharz, 1982; 1984a,b), influences of the environment (Lefebvre et al., 2007), and learned experiences during particular sensitive periods (Appleby et al., 2002) and throughout life. Fear-related behavior compromises performance, leads to significant numbers of dogs failing to complete training (e.g., Murphy, 1995; Batt et al., 2008), early withdrawals from working roles (Caron-Lormier et al., 2016), and can jeopardize dog and handler safety. Hence, amelioration of fear and anxiety is critical to maintain dogs in working roles and to ensure their well-being. Although current methods of selection and training are seemingly effective at producing many dogs which work in a remarkable array of environments, some dogs do not make the grade, and longevity of service is not always maximized. Programs should strive for optimal efficiency and they need to continually analyze the value of each component of their program, seek evidence for its value and explore potential evidence-based improvements. Here we discuss scientific evidence for methods and strategies which may be of value in reducing the risk of fear behaviors developing in the working dog population and suggest potentially valuable techniques and future research to explore the benefit of these approaches. The importance of environmental influences, learning opportunities, and effects of underlying temperament on the outward expression of fear and anxiety should not be underestimated. Identification of characteristics which predict resilience to stress are valuable, both to enable careful breeding for these traits and to develop predictive tests for puppies and procured animals. It is vitally important to rear animals in optimal environments and introduce them to a range of stimuli in a positive, controlled, and gradual way, as these can all help minimize the number of dogs which develop workinhibiting fears. Future research should explore innovative methods to best measure the relative resilience of dogs to stressful events. This could include developing optimal exposure protocols to minimize the development of fear and anxiety, and exploring the influence of social learning and the most effective elements of stimulus presentation.

Keywords

Fear, anxiety, prevention, working dog selection test, habituation

Introduction

Domestic dogs are used for a variety of working roles in which they are required to continue performing trained tasks in environments which may include novel, unexpected, or potentially aversive stimuli. Roles include herding, deterring predators, guarding, transportation, hunting, entertainment/sport (e.g., racing), contraband detection (e.g., police, military, and rescue services, Rooney et al. 2004), emotional therapy, and medical alert (Strong et al., 1999; Rooney et al., 2013). This diverse range of activities can expose dogs to potentially stressful stimuli in their surroundings: high levels of noise from machinery and gunfire explosions (and subsequent shock waves); transportation; variable terrain with unstable and varied surfaces underfoot; high levels of dust and smoke; loud machinery and traffic (Brown, 2011). In addition to this range of physical factors, dogs may need to cope with a variety of people, some behaving in unpredictable ways, the presence of other animals, and variable routines. Dogs can respond to these types of situations with behavioral signs of fear or anxiety, including freezing, withdrawing, or showing aggression (Casey, 2010). Such responses are likely to interfere with a dogs' ability to perform its role. In this study fear is defined as the behavioral response shown to actual danger (Boissy, 1998) and anxiety as the emotional state elicited in potentially threatening situations, for example, novelty, or when some elements of the environment predict a negative outcome (Ennaceur et al., 2006; Massar et al., 2011). Both affective states (fear and anxiety) are particularly concerning in roles for which optimal performance is critical for safety. For example, fear-related behaviors on the part of guide dogs or military search dogs can be life-threatening to both dog and handler and are therefore a common reason for failure in these roles (e.g., Murphy, 1995; Rooney et al., 2002; Batt et al., 2008).

The experience of fear is aversive (Grandin and Deesing, 2002), and by analogy with the experience of humans, it is likely that anxiety is similarly aversive because it too functions to promote avoidance of eliciting stimuli. Individual dogs which show fearful behaviors in a rehoming kennel environment appear to have an impaired ability to learn operant tasks (Blackwell et al., 2010), with similar effects demonstrated in a range of species (e.g., Shors 2004). This potentially makes training more time consuming and costly. Research has also suggested that high levels of fear-related behaviors can lead to increased disease risk (Terlouw et al., 1997) through modulation of immune responses, and possibly ultimately lead to a reduced lifespan (Dreschel 2010). Particularly relevant to dog programs with breeding schemes is the finding that decreased sperm quality is associated with anxiety in dogs (Memon, 2007). Fear and anxiety can inhibit complete erection and ejaculation (Kutzler, 2012, cited in Mills et al., 2014), and reduce proceptive and receptive behaviors in bitches resulting in a failure to breed (Grundy et al., 2002). Hence, in addition to welfare concerns arising from exposing working dogs to fear-provoking situations for which they are poorly prepared, and the issue of handler safety when dogs perform poorly, there are potential economic consequences. Fear and anxiety can lead to the loss of dogs from programs at assessment, during training, or later through early withdrawal from active working roles (Caron-Lormier et al., 2016), with an associated loss of the potential contribution they could make during their working lives.

Fear responses develop when animals are exposed to events or stimuli that they perceive as negative and salient (i.e., above their individual threshold of tolerance). On first exposure, animals will tend to show a startle response toward a novel stimulus and orientate toward it. This is an adaptive response to a change in their environment. On subsequent exposures, this response may either increase or decrease. Habituation is the process whereby a response gradually reduces with repeated presentation of the eliciting stimulus, and the threshold increases (Grissom and Bhatnagar, 2009). In contrast, sensitization is the process whereby an animal's response increases on presentation of a stimulus (Davis,1974). Having been sensitized to a stimulus and identified it as a threat, the animal will attempt to both identify predictors for the stimulus and develop a response to avoid it (e.g., to flee). In the working and companion dog, the aim is to manipulate factors both in the dog and its environment to maximize the chance that habituation occurs, while minimizing the risk of sensitization.

Multiple factors influence the development of behaviors associated with anxiety and fear across species and specifically in dogs. The nature of the stimuli and the manner in which it is first presented, including the social context, are both important. High intensity stimuli (e.g., explosions), or situations where aversive events occur consecutively without allowing animals a chance to recover, are very likely to provoke a response in most animals. Sensitization is more likely to occur when the presented stimulus is of high intensity or low predictability (Gray, 1971). Risk is increased where animals have had prior exposure to other negative events in their general environment or within the specific context. Individual differences in response thresholds mean that even seemingly benign stimuli will provoke a fear response in some individuals, a response that may be due to inherited characteristics, previous environmental influences, learned experiences, and to interactions between these effects.

Studies on personality may shed light on individual differences for the likelihood of animals developing fears. The “shy-bold” continuum has been demonstrated in a wide range of species (Jones and Gosling 2005). Murphree and Dykman (1965) developed a population of pointer dogs that showed extreme fear by selecting for those which reacted most to a range of standardized fearinducing tests. The relevance of the “shy-bold” continuum in dogs is supported by Svartberg and Forkman (2002) who identified 5 underlying factors of which 4 appeared to form a higher order factor analogous to the shy-bold characteristic.

If we simply test for the appearance of fear behaviors in an individual, we cannot be sure of the extent to which observed behavior is a manifestation of previous experience or underlying personality. What need to assess “resilience” to withstand stressful circumstances. Measuring contributing characteristics may be a promising approach to identifying risk factors for fear. Resilience (Yehuda et al., 2006a) is defined as the extent to which an individual is able to adapt in the face of adversity, trauma, or risk of threat. Coping strategies are well studied and have been defined in terms of approach (active or proactive coping) or avoidance (passive or reactive coping) styles (Roth and Cohen, 1986). In humans, active coping strategies improve individuals’ ability to manage stressful situations and reduce their risk of psychiatric illness (Valentiner et al., 1994). People who showed active coping strategies had lower levels of distress and post-traumatic stress disorder 6 months after the New York World Trade Center attacks (Silver et al., 2002).

Proactive and reactive types of responding have been well documented in laboratory rodents (de Boer et al., 2003) and in other species (see review, Koolhaas et al., 1999; but see also Forkman et al., 1995, and Jensen et al., 1995). Attempts have been made to characterize “coping styles” in dogs in rehoming centers, based on their physiological and behavioral responses to kenneling and their ability to learn an operant task. These responses suggest a “reactive” style of responding, or a more “proactive” style (Blackwell et al., 2010). A study of police dogs found 3, not 2, categories of dogs (Horvath et al., 2007). Coping responses are not immune to environmental influences or circumstantial changes in emotion, and individuals will often learn to show different behaviors in response to different situations (Roth and Cohen, 1986). In a working dog context, we may be inadvertently selecting for dogs with specific coping styles. However some coping responses, such as avoidance, may not be possible, and so we may also be causing stress by not enabling dogs to perform their preferred response.

Variation in coping styles suggests that the outward manifestation of the same inner emotional state will differ between animals. Some dogs may appear less overtly fearful than others because their coping strategy is more passive or reactive rather than proactive. This pattern presents a challenge for identifying signs of fear and anxiety when testing individuals and for deciding at what intensity to introduce new stimuli.

There are 3 potential approaches to reducing the risk of these fear- and anxiety-related behaviors developing in working dog populations:

a) select and breed individual dogs with the lowest risk of developing these behaviors,

b) control the environment in which dogs are kept and reared tominimize the risk of fear behaviors developing, and

c) optimize the method of initial stimulus exposure to minimizethe risk of dogs sensitizing and fears becoming established.

Most working dog agencies aim to achieve all of these, but they vary in their approaches. We describe some of the methods used and review evidence for their success. Possible practical solutions, such as improved selection, management, and training of working dogs, are also discussed, and questions raised about the value of some current practices.

Selection of animals most suitable for working environments

Working dogs are selected in a variety of ways. Most large assistance dog agencies have their own established breeding programs, while smaller organizations often procure puppies which may be selected on the basis of a suite of tests (with varying degrees of validation) and fostered before being reassessed for training. Some police, military, and security dog programs include breeding schemes, but a majority rely on purchasing adult dogs from private breeders or vendors. Some agencies procure unwanted companion animals from owners or rehoming centers. The Scandinavian military (Swedish and Norwegian) has well-established and validated methods for testing dogs before selection (e.g., Dog Mentality Assessment [DMA]: Wilsson and Sundgren 1997). Other selection tests vary considerably in validation criteria. Most working dog agencies have their own criteria for breeding and/or selecting animals with an assumed reduced risk of developing problematic fearand anxiety-related behaviors, especially fears of humans, loud noises, and novel environments. Criteria are implemented differently between programs and may involve selection of breeding stock, adult dogs, and/or puppies.

Testing for fearful behavior

Many agencies that train working dogs have developed tests to screen animals predisposed to show fearful behaviors. Details of the commonly used tests are often undocumented (e.g., UK military; Rooney et al., 2003). Some are published, including some of the tests used for guide dogs (Serpell and Hsu, 2001; Asher et al., 2013), police dogs (Slabbert and Odendaal, 1999; Svobodová et al., 2008), general service dogs (Wilsson and Sundgren, 1997), improvised explosives devices (IED) detection (Sherman et al 2015), and military dogs (Haverbeke et al., 2009; Sinn et al., 2010). Predictive testing is fraught with difficulties, and the extent to which this is objectively assessed is variable. There are several potential problems with many tests which need to be addressed.

Poor validity

Many tests have poor validity (Taylor and Mills, 2006). Single tests conducted in one context do not necessarily indicate behavioral responses in others. For example, tests of behavioral tendencies in rehoming centers show limited predictability of later owner reports of fearful and anxious behaviors in the home (Mornement et al., 2015). Tests routinely performed to select US military dogs, including tests for environmental and gun shyness, have been shown to have limited predictive validity when compared to later certification outcomes (Sinn et al., 2010). Haverbeke et al. (2009)

found that although some test elements seemed to predict later fear behaviors in Belgian military dogs, most tests were poorly predictive of later undesired behaviors. For many working dog tests, the predictive value cannot actually be calculated, as dogs which “fail” initial testing do not enter training and so no data are available on how these dogs would have performed compared to those that passed, and hence criterion validity cannot be ascertained (Taylor and Mills, 2006). More data should be collected on the relationship between tests and the outcome (e.g., validity), including for dogs that would ordinarily be rejected.

Study bias

Often studies which claim to reduce fear behaviors via selection tests concurrently change several procedures making it difficult to determine true cause. For example, Haverbeke et al. (2010) reported increased efficiency in military dogs which were selected specifically not to show fear of people, but they simultaneously introduced the Human Familiarisation and Training Program, which also increased levels of training for both dogs and handlers. The effect of selection based on exhibition of fear is therefore difficult to ascertain from outcome measures.

Presentation of high-intensity stimuli

Many selection tests involve presentation of sudden unexpected stimuli. The DMA (Wilsson and Sinn, 2012) used in Scandinavia presents a stranger, a dark room, loud metallic noises, an unstable surface, dummies, human “ghosts” and gunfiredall of which are potentially fear-provoking. These stimuli could sensitize dogs during testing, resulting in increased fear behavior on subsequent presentation (Davis, 1974). Because tests are conducted consecutively (Wilsson and Sundgren, 1997; Sherman et al., 2015), sensitization risk is increased (De Meester et al., 2011).

By observing the dog’s reaction to gradual exposure to specific stimuli which they are likely to encounter during operational activities, the level of exposure at which any contextually fearful response occurs is identified. This approach would allow treatment for specific fears, rather than outright rejection for dogs which may otherwise be suitable for some type of working life. Ogata et al. (2006) applied a Pavlovian aversive conditioning protocol and measured autonomic and behavioral parameters. Increased heart rate and body temperature occurred in response to a conditioned stimulus and suggested using fear conditioning as a method to test for trait fear (by measuring extinction of to the learned conditioned stimulus) in dogs. Behavioral changes did not consistently correlate with physiology and the authors concluded that autonomic reactions can be more reliable and consistent measures than some behavioral measures. Testing may benefit from the use of physiological measures, such as heart rate variability, shown to be associated with dogs’ behavioral responses to perceived stressors (Vincent and Leahy, 1997), and may prove more valuable than simple heart rate which was not seen to correlate with behavioral signs of fear and anxiety during working dog assessments (Sherman et al., 2015).

Lack of attention to fear behaviors during testing

In some organizations, testing focuses on perceived priority working traits such as motivation to work and exhibition of trained attack behaviors. Behavioral signs of anxiety or fear are seldom recorded. The selection of confident dogs is a major priority for many agencies, but selection tests may not effectively select against fear. Objective video analysis of Swedish military selection tests revealed higher levels of fear among dogs which were selected compared with those that were not (Foyer et al., 2016), suggesting inadvertent selection for some fearful behaviors. Observers vary in their ability to accurately interpret behavioral responses, especially if signs are subtle (Tami and

Gallagher, 2009; Marti et al., 2012). Accurate and early detection of behavioral indicators of fear is critical and resources to teach observers to recognize the commonly missed signs have been developed (Loftus et al., 2012). Such tools will likely be useful in conjunction with physiological indicators of behavioral reactivity.

Reliance on testing puppies

Organisations that breed or purchase puppies wish to reject puppies least likely to succeed as early as possible. Many agencies use a suite of "puppy tests" which try to predict fearfulness among other traits. Tests for fear of loud noises include using weights dropped in buckets (Murphree and Dykman, 1965) or on tables (Svobodová et al., 2008), whistles (Pfaffenberger and Scott, 1976), metal cans (Fisher and Volhard, 1985), party poppers (Hoffmann et al., 1995), simulated thunderstorms (Seksel et al., 1999), clapping (Champness, 1996), or pistols (Slabbert and Odendaal, 1999). Tests may examine fear of novel people (e.g., Murphree and Dykman, 1965; Slabbert and Odendaal, 1999); novel objects, including umbrellas (Hoffmann et al., 1995), hair driers (Seksel et al., 1999), novel environments (e.g., open stairs [Goddard and Beilharz, 1986]), and unstable surfaces. A major flaw of testing at a very young age is that it does not account for modification by ontogeny and learning. Analysis of the test commonly used by Australian Guide dogs (Goddard and Beilharz, 1982; 1984a,b; 1985; 1986) revealed that although fearfulness as an adult was significantly predicted by a test at 8 weeks of age (and was the only predictable trait), the predictive value increased with age of testing. In contrast, a large meta-analysis of 31 "personality" tests in dogs (Fratkin et al., 2013) found that puppy behaviors were moderately consistent even with longer intertest intervals but that fearfulness (along with responsiveness to training) in puppies was one of the least consistent dimensions.

Scott and Bielfelt (1976) saw no increase in the adult performance of guide dogs when breeding stock were selected on the basis of their puppy test scores. This finding is supported by a study of the development of Arms and Explosives search dogs (Rooney et al., 2003), which showed little correlation between behaviors shown by 8-week-old puppies and those seen in equivalent tests in adult dogs. Instead, an inverse correlation between fear of slippery surfaces at 8 weeks and 11 months was found. Similarly, Asher et al. (2013) found that puppies which scored as more confident on a ramp (1/5 stimuli tested) were less likely to qualify as guide dogs. These discrepancies may be partially due to using deficiencies highlighted in the puppy test to direct remedial training. Some puppy tests detect predispositions that can subsequently be overcome by operant conditioning (e.g., puppies finding their existing behavior "unsuccessful" and changing to an alternative strategy). The variable and generally low degree of success in such tests suggests that trying to predict adult behavior from puppy tests is problematic, and as failures at procurement tests are rarely trained, the true test reliability is not assessed. The value of puppy testing is therefore questionable without assessment of predictive validity.

The influence or prior learning as well as temperament

Many tests purport to measure "temperament" but actually measure a dog's behavioral response to a specific contextual challenge. Such contextual tests may be desirable (e.g., to detect whether dogs being rehomed show aggression when handled), but it is potentially more problematic where tests are screening breeding animals. The approach cannot separate a response due to a particular historical aversive experience from an underlying predisposition (e.g., "temperament"). It is unlikely that the "temperament" and "learned" component of a behavior in an individual dog can be teased apart, it is important that the different contributions of these elements are understood to enable tests to be developed for different requirements.

When using an open-field model to assess sound-induced fear and anxiety in potential IED dogs, Gruen et al. (2015) exposed animals to loud noises on consecutive days but did not explore differences in the dogs' past experiences or exposure to noises. In the same population of dogs, Sherman et al, (2015) saw differences between individuals in their "emotional reactivity" and the dogs showed consistency in individual response across a large number of stimuli. Such responses may be affected by both personality and past history. The degree to which the responses predicted working ability in operational conditions and the relative importance of genetic versus experiential factors is unknown. To detect dogs that are at risk for developing fear and anxiety, rather than assessing preexisting fear, one could instead test for variance in underlying decision-making processes that influence resilience to stress exposure (Yehuda et al., 2006a). Understanding whether dogs exhibit "proactive" or "reactive" styles of responding (Koolhaas et al., 1999) may also help to identify the nature of their response to fear-evoking stimuli. Other approaches could include measuring the relative degree of "optimism" and "pessimism" (cognitive bias). Animals that have a more "pessimistic" cognitive bias tend to judge novel or ambiguous stimuli as "more likely to be negative" and so may be more likely to develop fear responses. A "pessimistic" cognitive bias has been associated with a tendency to show separation-related behavior (Mendl et al., 2010) and is reduced by treatment with a selective serotonin reuptake inhibitor, fluoxetine (Reconcile), combined with behavior modification (Karagiannis et al., 2015). Because cognitive bias appears to be a better measure of underlying mood state rather than more transient emotional responses (Mendl et al., 2010), it may be a better predictor of behavioral responses across contexts and a valuable tool in predicting success in working environments.

Research into methods by which to better predict behavioral tendencies and predictors of resilience is required and to better understand the extent to which underlying temperament characteristics, themselves, become plastic with environmental changes and stress exposure (Yehuda et al., 2006a). It may be beneficial to investigate whether variation in underlying characteristics of temperament correlate with patterns of allelic variation in genomewide association studies (Hayward et al 2016) or in the expression in targeted areas of the genome.

Several novel measures have been associated with increased resilience to stressors in laboratory species and humans (Yehuda et al., 2006a). Neuropeptide Y (NPY) may have an important role in reducing risk through modulatory effects on important regulatory systems in the brain such as the hypothalamic-pituitary adrenal (HPA) axis (Heilig, 2004), and noradrenaline release (Pich et al., 1993). NPY has been found to be higher in human military service veterans who have been exposed to extreme stress but did not develop PTSD, when compared with those who developed PTSD. NPY also appears to correlate with the extent of symptom improvement (Yehuda et al., 2006b). Such assays may be biological measures of resilience and/or ability to recover. The ratio of plasma dehydroepiandrosterone sulphate to cortisol was positively correlated with peoples' ability to perform well in stressful military situations (Morgan et al., 2004). Other potential markers of resilience associated with differences in HPA axis activity include 24-hour urinary cortisol secretion, circadian rhythm of cortisol release, lymphocyte glucocorticoid receptor number, and lysozyme IC50 as an indicator of enhanced glucocorticoid responsiveness (Yehuda et al., 2006a). Investigation of some of these new measures, either in isolation or through metabolomic profiles may indicate dogs most likely to withstand the stress of working dog life.

Measures of patterns in sweat production that are linked to emotional responses such as fear can be obtained noninvasively in humans (Lin et al., 2011) and sheep (Reefmann et al., 2009a,b). Measurement in the dog would require modification because canine apocrine gland distribution differs.

Studies of “laterality” (preferential use of one side of the body) suggest possible links with guide dog success (Batt et al., 2008), specific aspects of guide performance (Batt et al., 2010), general problem solving ability (Marshall-Pescini et al., 2013), and some measures of fear, although not with agility competition success (Siniscalchi et al., 2014). In humans, an association between hand preference and the propensity to experience anxiety has been described, and similar patterns identified in dogs. Dogs with weaker paw preferences (a measure of cerebral lateralization) were more reactive to thunderstorm and firework noise (Branson and Rogers, 2006). Motor lateralization, combined with other factors, was indicative of success in potential guide dogs (Batt et al., 2008). Different measures of laterality and different ways of interpreting measures often yield differing results, so use of laterality measures for other working disciplines requires further testing.

Breeding against propensity to show fearfulness

Several breeding experiments have suggested that fearful behavior is heritable (Murphree and Dykman, 1965; Goddard and Beilharz, 1982). The distribution of behavioral phenotypes of subsequent generations has been changed by selecting breeding animals of extreme “types” at either end of a continuum (i.e., the most and least fearful). These studies predominantly concentrate on social fearfulness, although several also look at nonsocial stressors including reactions to loud noises. It may therefore be possible through selection to reduce the frequency of the more extreme phenotypes of fearful behaviors. The breeding program for UK guide dogs has been effective in reducing the occurrence of fearassociated behaviors (Willis, 1995) and many breeding programs have similarly bred against outward expression of fear.

Studies using working dog populations (e.g., Hsu and Serpell, 2003, Arvelius et al., 2014a,b) have similarly suggested that the expression of fearful behaviors is partly heritable. In the Swedish armed forces, the behavioral scores for confidence and environmental sureness as measured during the DMA had heritabilities of 0.23 and 0.15, respectively (Arvelius et al., 2014a) (see also Wilsson, 2016, this volume). Heritability assesses the proportion of the variation in the populations’ test scores that can be accounted for by the genetic component. When assessing rough collies, Arvelius et al. (2014b) found similar heritabilities for test scores and relatively high correlations between fearful behavior during testing and that reported by owners using the Canine Behavioral Assessment and Research Questionnaire (Hsu and Serpell, 2003). Arvelius et al. (2014b) concluded that if breeding animals were to be selected on the basis of scores for curiosity/fearlessness in the DMA, then breeders would also report a concurrent decrease in nonsocial fear scores. When examining general fearfulness, 1 of 3 fear-related factors derived from multivariate analysis of multiple subtests used on potential guide dogs, Goddard and Beilhraz (1984) obtained heritability estimates as high as 0.80.

Selection based on test scores should be considered with some caution because little is known about the specific underlying characteristics selected for within breeding programs based on behavioral phenotypes. It is unknown, for example, whether dog populations show the type of structure suggested by Wilson et al. (1994) -shy-bold characteristics where animals at both population extremes show low environmental flexibility. If this were the case, then selection against “innately shy” (or fearful) dogs may not just alter the proportion of dogs which exhibit fear, but also the proportion which are flexible in their response across environmental conditionsda disadvantage in a working animal needing to adapt to a range of environments. It is unknown whether such selection acts at the level of the animal’s perception of fear-inducing stimuli, the central evaluation of stimuli and assignment of emotional significance, their expression of fear behaviors, or even the animal’s ability to inhibit these behaviors. By selecting against dogs which show an active fear response (e.g., for those whose behavior changes little when presented by stimuli), we may be selecting against "proactive" characteristics and favoring more passive or "reactive" fear responses (Koolhaas et al., 1999). Dogs with a "passive" style of responding may have a different strategy for achieving rewards and avoiding aversive events, with consequent implications for training success. A less obvious behavioral response to a stimulus may not necessarily indicate a reduced negative emotional experience. It is possible that selective breeding programs may in fact result in unreactive "zombies" (D'Eath et al., 2010).

Behavior traits may be genetically linked to other morphological, or physiological characteristics (e.g., Mackenzie et al., 1985), which may be undesirable. Hence, although it is theoretically attractive to select animals from which to breed offspring with a reduced risk of showing fearful behaviors, current knowledge about the characteristics which underlie risk of behavioral signs, their patterns of inheritance, and their association with allelic variation in genome-wide studies, is currently insufficient to determine the consequences of such an approach. It may be that a more suitable approach is to investigate the heritability of underlying characteristics such as stress resilience and optimism, rather than selecting directly for phenotypic presentations of fearful behavior.

Reducing the risk of fear-related behaviors through environmental manipulation and rearing

Preventing problems is ultimately more cost-effective than managing them, so it is equally important to focus on the aspects of the environment that may influence the development of fearful responses. There are also opportunities to control both prenatal and postnatal maternal environment when there is considerable synaptic plasticity, and appropriate actions can ameliorate the chance of later problems developing.

Manipulation of factors affecting susceptibility to stress and hence likelihood of developing fears

Background levels of arousal and concurrent causes of fear and anxiety during stimulus presentation can increase the risk of sensitization (Davis, 1974). There is considerable evidence that states of anxiety in rodents increase the magnitude of the startle response (Bijlsma et al., 2010). People with anxiety disorders are more likely to develop a range of conditioned fear responses (Lissek et al., 2005). Therefore, adapting living environments, human contact, and general training methods to avoid anxiety-provoking situations is important, both for home-living and kenneled working dogs. Detailed reviews exploring ways to minimize stress of kenneled dogs are available (e.g., Gaines, 2008; Rooney et al., 2009).

General human contact

Interactions between dogs and human handlers are important and the interspecific relationship may affect numerous aspects of social behavior, including the development of fears. Human interaction can be beneficial to dogs in reducing the stress of confinement (Coppola et al., 2006) and may increase indicators of positive well-being, such as decreased blood pressure and increased bendorphin (Odendaal and Meintjes, 2003). A program of human interaction in chronically stressed shelter dogs resulted in reduced excitation and cortisol in the experimental dogs compared to controls (Hennessy et al., 2006). Handlers of military dogs who took their dogs home after work described them as less fearful, more sociable, more accepting of stroking by strangers, and less likely to bite compared to those handlers whose dogs remained in kennels (Lefebvre et al., 2007). Pet dog owners who shared more activities with their dogs were less likely to categorize them as "nervous" (Bennett and Rohlf, 2007), although cause and effect are unclear.

Reduction of stress during routine training

The type of contact dogs receive is important. Data from Hiby et al. (2004), Blackwell et al. (2008), and Rooney and Cowan (2011) strongly suggest that punishment-based methods may increase levels of fear and anxiety and potentially lead to sensitization to other environmental stimuli. Punishment-based training of pet dogs is linked to an increased incidence of behavior problems (Hiby et al., 2004), including fear, anxiety and aggression (Blackwell et al., 2008), wariness towards strangers, reduced playfulness (Rooney and Cowan, 2011), and increased anxietyrelated aggression and excitability (Arhant et al., 2010). Dogs regularly trained with electric shocks show behavioral evidence of fear and distress in the presence of their owner, even outside the training context (Schilder and van der Borg, 2004). Dogs subjected to physical reprimands have been shown to score significantly higher for aggression (Hsu and Sun, 2010) and dogs whose owners report using a higher proportion of punishment are less likely to interact with a stranger (Rooney and Cowan, 2011), which could be due to fear and anxiety.

Most working dogs are now being trained primarily using positive reinforcement and negative punishment. There are still some trainers who use coercive approaches based on a "dominance" approach to interpreting dog behavior (Bradshaw et al., 2009), and rough handling remains common in specific disciplines and organizations (Haverbeke et al., 2009). Aversive methods, including pulling the leash and hanging, are associated with low body postures indicative of fear or distress and poor performance in military working dogs (Haverbeke et al., 2008). Those military dogs suspected to have been handled roughly in the past were perceived to be more fearful (Lefebvre et al., 2007). During protection and obedience work, dogs which had received more punishment tended to show more fear behaviors (Haverbeke et al., 2009).When examining prison officers' behavior toward their search dogs, Rooney et al. (2007b) found that handlers who believed in using high levels of punishment tended to have less confident dogs. All studies discussed are correlational, and the possibility exists that dogs with specific problems may be more likely to be trained using coercive methods. Regardless, such dogs show a consistent pattern between aversive training methods and behavioral problems, including fears. Longitudinal cohort studies and experimental trials would be needed to demonstrate causal links.

Avoiding positive punishment-based techniques and interacting with dogs in a calm and consistent way is likely to be important in reducing the development of fear-related behaviors. Horses trained using positive reinforcement interacted more with the handler (Sankey et al., 2010) and, zoo rhinos (Holden et al., 2006) and primates (Savastano et al., 2003) have been successfully trained to present various body parts in a calm safe manner through operant conditioning using positive reinforcement. This approach is used in farm environments for ease of handling, reduction of stress (Kilgour et al., 1991), and cognitive enrichment (Manteuffel et al., 2009). In military dogs in which positive reward training sessions were used in conjunction with altered selection procedures and handler training, dogs demonstrated increased confidence and overall performance (Haverbeke et al., 2010), suggesting that training methods are vital in working dog operations.

Kennel management and routines

Gaines (2008) stresses the importance of husbandry regimes to decrease stress of kenneled dogs. Specific husbandry processes that can lead to distress include shutting dogs in small dark inner kennels while cleaning their home kennel (Gaines, 2008), using noisy equipment such as cleaning hoses (Mills, 2005) and separating dogs from conspecifics (Walker et al., 2014). Identification and amelioration of these concerns is important to stress reduction. Increasing the predictability of a dog's routine may be important (e.g., walking and feeding at predictable times) if rigidity which may limit future flexibility is avoided (Gaines et al., 2008). To best prepare dogs for a range of situations and activities, gradual adaptation from initially predictable routines to less predictable and more variable schedules may best be done over time. In this way, dogs will "have an expectation of the unexpected" and may cope better with changing circumstances when they become necessary.

Early-life environments

Prenatal and postnatal effects

Those programs with breeding and puppy-walking schemes can affect adult dog behavior by influencing prenatal and postnatal environment. Most work on early-life effects has focused on human development (e.g., Grant et al., 2009; Hellemans et al., 2010), rodents (Bosch et al., 2007), and farmed species (e.g., pigs, Jarvis et al., 2006; sheep, Dodic et al., 2002) and has shown that prenatal anxiety adversely affects stress reactivity in offspring. In comparison, very little is known about the effect of prenatal experience on the behavior and development of puppies. Given the compelling evidence in other species, the ability of pups to recognize odors learned in utero (Wells and Hepper, 2006), and similar endocrine profiles and changes in cortisol in pregnant bitches as in other mammalian species (e.g., sheep; Concannon et al., 1978), it is likely that dogs are similarly influenced by variation in maternal exposure to stressors during pregnancy. It would seem prudent that due attention is given to the range of experiences of breeding bitches during pregnancy (Gaines et al., 2008).

There is evidence that puppies manipulated in the neonatal period differ in later stress responsiveness. Battaglia (2009) describes a study on a biosensor program for military dogs where puppies of 3-16 days of age were stimulated daily using a range of handling techniques, including being placed on a cold towel. Puppies had improved cardiovascular function (HR), stronger heart beats, stronger adrenal gland function, greater tolerance to stress, and greater resistance to disease (Battaglia, 2009). Mild stressors applied in early life have been reported to promote resilience (Macrì and Würbel, 2006, 2007; Macri et al., 2009; 2010; 2011). Providing pregnant mothers and neonatal puppies with a stimulating but not overtly fear-inducing environment may promote resilience in puppies. Mixing bitches into new social groups, unaccustomed confinement or social isolation (Rooney et al., 2007a), malnutrition (Poore et al., 2010), inconsistent handling, transport, and exposure to specific inescapable ear-eliciting situations (e.g., loud noises) should be avoided.

Management of weaning

In most "natural" environments, the process of weaning is a gradual one, with offspring becoming progressively more emotionally and nutritionally independent (Latham and Mason, 2008). Many companion animals, including dogs, are removed suddenly from their mothers, usually concurrently from their familiar social and physical environment. Behavioral (Houpt and Hintz, 1983; McCall et al., 1985), and physiological (McCall et al., 1987; Malinowski et al., 1990) signs of distress at this sudden weaning are observed in many species. Activation of the HPA axis has also been shown to continue for several weeks after weaning in both rodents and primates (Levine, 2000), and there is no reason to assume this is not the case for dogs.

Appropriate management of weaning can reduce the risk of inducing fears. The nature and period over which maternal separation occurs can have positive or detrimental effects on later resilience to stressors. Rodents studies show that early separation of pups from their mother can induce short- and long-term changes in the stress reactivity system, as indicated by the potentiated HPA axis response to subsequent stressors (e.g., Knuth and Etgen, 2007; Lippmann et al., 2007). Early and abrupt separation also enhances manifestations of anxiety and depression-like behaviors (Fabricius et al., 2008; Lambás-Señas et al., 2009) and impairs spatial learning and memory (Aisa et al., 2007; Tata et al., 2015) during adulthood. In contrast, repeated, short maternal separation increased resilience to stressful events later in life (Parker et al., 2006; Benetti et al., 2007) and reduced fearfulness (Macrì and Würbel, 2006). The positive effects of such separations may have resulted from increased attention from the mother when the pups were returned. Increased maternal stimulation appears to have a beneficial effect on neural development and can reverse the effects of early-life stressors (Imanaka et al., 2008). Slabbert and Rasa (1993) studied South African Police dog puppies which received equal human attention but were separated from their mothers at either 6 or 12 weeks. The late weaned puppies gained weight more quickly and were in better condition. The study did not report any differences in fear behavior toward people or other stimuli.

Evidence of optimal weaning protocols for working dogs is limited. Research suggests that protocols should vary across breeds because they differ in the age of onset of fear-related behaviors (Morrow et al., 2015). The advantages associated with maternal care, social interaction with littermates, learning social signaling, and avoidance of sudden traumatic events all support later and gradual weaning of puppies destined for working environments. Regardless, many organizations routinely wean early to ensure socialization opportunities are maximized and to facilitate foster placement. For example, in the United Kingdom, Guide Dogs for the Blind routinely remove puppies from their mother at 6-8 weeks, below the age generally advised for other dogs. The relative quality of breeding and rearing environments of pet dogs affects the ideal weaning time (Appleby et al., 2002), a finding applicable for working dogs.

Introduction of potentially fear-provoking stimuli in an optimal way

Habituation

The relative risk of habituation or sensitization will vary with characteristics of the stimulus, the personality of the dog, and the state of the individual animal at the time of stimulus presentation. Controlling all these factors is important to minimize the chances of a working dog developing fear-associated behaviors. Habituation protocols are commonly used by working dog programs aiming to introduce dogs to stimuli to which they will be exposed when working.

Evolutionarily relevance (e.g., Mineka and Ohman, 2002), suddenness (Trimmer et al., 2008), predictability (Schalke et al., 2007), intensity (Winslow et al 2002), frequency of exposure of the stimulus (Gotz and Janik, 2011), the ability of the animal to control exposure (Levine, 2000), and its current emotional state (Davis, 1974) will all affect the probability of an animal experiencing sensitization. Mills et al., (2014) identified important aspects of a stressor for dogs to be physical characteristics (affective quality, intensity, magnitude, duration), the predictability, and expectation of the animal in relation to the stressor, and the preparation given to enable coping and opportunities for control over the stressor.

Initial intensity of stimulus presentation needs to be below the threshold of startle for the animal. In general, the principle is to start as low as possible because it is better to increase the level gradually from well below all dogs' tolerance than risk sensitization. Because each animal will have a unique tolerance to different types of stressors, it is important to select an initial level of exposure which is below that to which any individual in the program population will respond aversely. In a diverse population of procured dogs, it may be difficult to find a level to which no dogs respond. A preliminary test to identify those outlying individuals with established fears is recommended. These dogs would then require remedial training including desensitization, counterconditioning, prior to controlled exposure, and habituation.

The benefits of this type of gradual introduction to novel stimuli in contrast to repeated exposure of a full intensity stimulus (“flooding”) are understood by many animal trainers. Horses given a gradual introduction to a moving white nylon bag showed both behavioral and physiological differences compared to those without such an introduction. Those with gradual exposure protocols needed fewer training sessions to remain calm on stimulus presentation, and all subjects in the gradual exposure group completed training, which was not the case for those in the full exposure group (Christensen et al., 2006). Sixteen IED dogs showed an overall reduced response to noise over a 5-day testing period, but as the noises presented on each day were different, it is likely that the dog habituated to the testing environment. Some dogs did not decrease in responsiveness which suggests that the initial exposure level may have been too high (Gruen et al., 2015).

Habituation to noise in rats is more effective when exposure is interspersed by longer gaps. Rats were exposed to either six 30minute sessions of white noise in a single day or one 30-minute exposure daily for 6 days (Masini et al 2008). The groups showed similar habituation of plasma corticosterone, ACTH, heart rate, and core body temperature, but the habituated responses were not retained in the single-day exposure group, on reexposure 48 hours later. The effect may be associated with the experience of rapid eye movement sleep between training sessions (e.g., Levin and Nielsen, 2007) which consolidates memory. Habituation sessions may benefit from being separated by a period of sleep.

Habituation in laboratory rodents is context specific, so “recovery” of the response occurs in contexts different from that where the habituation occurred (Jordan and Strasser, 2000). Should elements of the context alter, a startle response may recur, particularly if the change of context is salient to the animal. A dog may habituate to a noise presented in one situation but may react when this is presented at the same intensity in a new context (Leiner and Fendt, 2011). This effect is influenced by the extent to which the “new” context is familiar or novel for rats. Behavioral responses reoccurred when a habituated stimulus was presented in a novel context, but not when the animal was familiar with the context (Nyhuis et al., 2010). Prior habituation to environments in which stimuli will be presented, such as introducing dogs to the environments in which potentially frightening stimuli may occur, may aid with the process of habituation and help prevent fears developing. Many working and performance dog organizations do not use this gradual approach. Initial greyhound schooling generally happens in an unfamiliar location, in the presence of new people, and dogs may have been transported in a new vehicle to the schooling location.

The presentation of numerous potential stressors simultaneously may lead to sensitization in some individuals (Rooney 2012; Cobb et al., 2015). Working dog programs often use screening tests which present stimuli at full intensity. When adult dogs are procured from different sources, they may have widely different background experiences. Dogs that have previously learned a fear response may display their learned response at a very low level of stimulus presentation. For others, test scenarios may be entirely novel and potentially risk sensitization due to exposure to multiple novel stimuli in a novel environment. Pretesting with gradual exposures to ascertain the levels to which dogs in the population respond for each potential stressor is important.

Social context affecting fear responses

Conspecifics

The potential for dogs to be influenced in their development of fear by the presence and behavior of other dogs is important. This has implications both for the context in which dogs should be tested for their propensity to exhibit fear and anxiety, and for the conditions under which dogs should be introduced and habituated to potentially fear-inducing stimuli. It is commonly reported that if a dog first encounters a new stimulus in the presence of a fearful dog, it is more likely to develop fear itself (e.g., Landsberg et al., 2012). Introduction in the presence of a previously conditioned and calm dog may reduce the likelihood of a fearful response. Trainers use the presence of calm unreactive conspecifics as a means of reducing fear in “nervous” animals, but the potential for using a calm “demonstrator” dog in working scenarios needs to be evaluated.

Human contact

Human interaction during stressful events can also exert a calming effect. Twenty minutes of gentle stroking inhibited the immediate increase of cortisol during a venipuncture procedure (Hennessy et al., 1998). Humans generally have greater success at inhibiting stress responses to a novel environment in dogs than do familiar dog companions (Tuber et al., 1996).

Some human interaction can be detrimental and increase the likelihood of fears developing. The saying that fear “travels down the lead” acknowledges that anxious owners and handlers can increase the likelihood of dogs reacting fearfully. Levels of cortisol increased in agility dogs when their male owners became angry after losing a competition (Jones and Josephs, 2006). Inexperienced owners were more likely to describe their dogs as showing fear of traffic, loud noises, and other dogs (Jagoe and Serpell, 1996), suggesting that fear and anxiety may result from owners lacking the knowledge to respond in an appropriate and helpful way. Dogs may also be influenced by the behavior and personality of their owner. Tense and emotionally less stable owners may be more likely to own aggressive dogs (Podberscek and Serpell, 1997). Aggression in dogs may be associated with fear, so it is possible that owner personality affects the way the person behaves toward and interacts with their dog, which may affect the dog’s propensity to be fearful (Podberscek and Gosling, 2000). Dreschel and Granger (2005) noted that owners scoring highly for anger/hostility, depression/ dejection, and fatigue were less likely to interact with their fearful dog during a simulated thunderstorm, although there were no significant effects of owner behavior on the dogs’ behaviour. Interaction with dogs during fearful episodes has not been traditionally recommended as it may inadvertently reward and thus reinforce fearful responses. A survey of dog owners in New Zealand found an association between owners comforting their dogs and increased severity and duration of fear behavior over time (Dale et al., 2010). Such associations may not be causal, and the advice against comforting fearful dogs has been challenged because the reinforcement may be of the coping response rather than the fear itself (Casey, 2013). Calm, informed, and diligent handlers and trainers are critical to any effective fear-reduction program.

Introduction of puppies to potential stressors sensitive period of learning (“socialization period”)

In programs that breed or rear puppies, appropriate early introduction to potential stressors is important. The period of development and maturation of senses lasts until about 1214 weeks, when puppies become more independent. This is an important time of enhanced synaptic plasticity, where exposure to environmental stimuli has a profound influence on later behavior (Casey and Bradshaw, 2008). Using this period to ensure that puppies have had positive experiences of stimuli and situations they are likely to come across in their adult lives is commonly acknowledged to reduce the risk of fear-related behaviors in companion (Appleby et al., 2002; Howell and Bennett, 2011) and laboratory (Boxall et al., 2004) dogs. Dogs should be introduced to the types of situations and stimuli they will encounter in adult life before 3 months of age (preferably by 8 weeks and certainly no later than 12 weeks; Scott and Fuller, 1965). Puppies are curious about novel situations, but they can readily develop fear behaviors early in their exposure periods. Studies on research dogs have shown that handling for 3 minutes per day and exposure to stressors (e.g., changes in ambient temperature, different flooring, different handlers) in a manner that gradually increases in intensity and duration has positive effects on resistance to disease, emotional reactivity, and problem solving (Meunier, 2006). Gazzano et al. (2008) reported that well-handled puppies of various breeds were calmer. Presentation of video images of animate (e.g., people, dogs) and inanimate (e.g., traffic, vacuum cleaner) stimuli to puppies was associated with a reduction in later fear behaviors (Pluijmakers et al., 2010). Such potential interventions can be explored in working dog rearing programs.

Many working dog breeding establishments (e.g., USA Transport Security Administration; Thomas, 2011) use this sensitive period of development to introduce dogs to a variety of stimuli including other animals, people, children, noises, slippery surfaces, and unstable floors. When considering socialization programs and exposure to potentially frightening stimuli, it is important to control exposure and the level of stimulus, increasing intensity gradually to avoid sensitization and inadvertent creation of anxiety or fear responses. Gradual habituation to an indoor crate, followed by an outdoor kennel environment, reduced physiological stress levels when search dogs entered military training kennels (Rooney et al., 2007a). As risk of sensitization is increased in animals experiencing concurrent stress, the experience of kenneling, handling, and enrichment also needs to be addressed.

Puppy socialization classes are often used to exposure pet or companion dogs to stimuli which commonly cause fear reactions in adult dogs. Stimuli may include social situations, fireworks and thunder (Blackwell et al., 2013), and urban noises such as engines (Sherman and Mills, 2008). Population-based studies have had produced mixed results about the potential benefit of such classes on later behavior. Four weekly puppy socialization classes were successful in training companion puppies but produced no significant change in responses to social stimuli 4-6 months later (Seksel et al., 1999). Similarly, 5 socialization sessions did not affect the success rate of guide dog puppies (Batt et al., 2008). However, large scale population surveys have identified positive effects, and dogs that attended puppy socialization classes were less likely to be reported to show undesirable reactions to other dogs (Blackwell et al., 2008). Attending puppy classes was associated with a protective effect against owner-reported aggression (Casey et al., 2014). The methods used in these classes are variable and the extent to which they may contribute to reducing fear will depend on stimuli presentation method and attentiveness to the dog’s behavioral responses.

There have been several studies on early-life experience in working dogs. Goddard and Beilharz (1984) examined fearfulness and activity/distraction in 102 potential Guide Dogs. When the quality of puppy-walking experiences was rated and compared to later behavior, the most significant correlations were between high-quality puppy walking and decreased fear of unusual objects (Goddard and Beilharz, 1985). Rooney et al. (2003) found that compliance of puppy walkers with a program of introduction to new experiences was a major factor in determining the later success of specialist search dogs. Programs that provide gradual, early introduction to stimuli for working dogs, combined with optimal weaning, may be beneficial and should be investigated further. Play and relaxed social interactions provide ideal contexts for puppies to encounter stimuli and learn that they are not threatening. Slabbert and Rasa (1997) used observational learning to demonstrate that pups, who were allowed to stay with their mothers and observe them in a search and retrieve task, were better at completing the same task (without reinforcement) than nonexposed pups at 6 months. The behavior of the mother could potentially be very powerful in influencing the behavior of her offspring and may be a potential area for research in the reduction of fear responses.

The number of potential stressors is great in working environments. For example, search and rescue or military search dogs may be exposed to explosions in which loud noise, shock waves, and dust are experienced simultaneously. Little evidence exists as to the optimal timing of stimulus exposure in early life, whether composite stimuli need to be broken into their component parts and separately presented, or whether puppies will tolerate combined stimuli at low intensity. Because of the importance of this period in influencing adult life across species (Casey and Bradshaw, 2008), we can be confident that controlled exposure will be beneficial. Agencies which breed puppies for working roles should carefully consider policy in this area, and those which use regular suppliers should encourage gradual controlled exposure.

Treatment

Following evidence-based selection criteria, manipulating the living environment, and providing opportunities for learning will help to minimize the number of working dogs which develop fears and anxiety. Some fear responses remain inevitable when animals are working and training in unpredictable and potentially dangerous environments. Most of the fear-related behaviors in USA military working dogs were reported to be acquired as adult dogs (Burghardt, 2003). There is value to developing effective treatment protocols for dogs showing fear responses -for animals that demonstrate preexisting fear responses at procurement, before training that may then interfere with ability in training and for dogs already in training, in service or returning from operations, which have developed fear responses. Any treatment should include a full medical and behavioral workup of the animal to ensure there is no underlying medical condition. This examination should be followed by investigation of stimuli that elicit and maintain the dog’s fear response and a decision about how best to restore the animal’s well-being. Such investigations may lead to some dogs ending their working career, but many dogs may be able to continue working much longer than if they were untreated. Behavior modification programs based on desensitization and counter-conditioning (e.g., Levine et al., 2007), a combination of presenting an animal with the fear-eliciting stimulus at an initially low level, and positively rewarding the animal for showing an alternative (calm) response to that shown previously, are vital.

Conclusions

Propensity to show anxiety and specific fear responses results from an interplay of individual differences in animals and learning throughout life. Because the development of fearful behavior is a major reason for working dog failures, attention to methods likely to minimize fear in working populations is critical. Programs should strive for optimal and improved efficiency to reduce the wastage of dogs. Program managers should continually analyze the value of each component of their program, for puppies and adult dogs, to seek evidence for its value and explore potential evidence-based improvements. We have discussed valuable techniques already used and suggested important areas for future research effort.

The development of fearful behavior has some heritable component, but the environment and past learning also have effects. Identification of characteristics which reliably predict resilience to stress, careful breeding for these traits, the derivation of predictive tests for puppies and procured animals, rearing animals in optimal environments, and introducing them to a range of stimuli in a positive, controlled, and gradual way can help minimize the number of dogs which develop work-inhibiting fears. Future research should explore innovative methods of best measuring the relative resilience of dogs to stressful events and develop optimal protocols to enhance such resilience.

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Conflict of interest

The authors declare that there are no conflicts of interest involving any of the authors and this work. Rachel Casey has started working at the UK charity Dogs Trust since the submission of this article.

References

Aisa, B., Tordera, R., Lasheras, B., Del Río, J., Ramírez, M.J., 2007. Cognitive impairment associated to HPA axis hyperactivity after maternal separation in rats. Psychoneuroendocrinology 32, 256e266.

Appleby, D.L., Bradshaw, J.W.S., Casey, R.A., 2002. Relationship between aggressive and avoidance behaviour by dogs and their experience in the first six months of life. Vet. Rec. 150, 434e438.

Arhant, C., Bubna-Littitz, H., Bartels, A., Futschik, A., Troxler, J., 2010. Behaviour of smaller and larger dogs: effects of training methods, inconsistency of owner behaviour and level of engagement in activities with the dog. Appl. Anim. Behav. Sci. 123, 131e142.

Arvelius, P., Eken Asp, H., Fikse, W.F., Strandberg, E., Nilsson, K., 2014. Genetic analysis of a temperament test as a tool to select against everyday life fearfulness in Rough Collie. J. Anim. Sci. 92 (11), 4843e4855.

Arvelius, P., Strandberg, E., Fikse, W.F., 2014. The Swedish Armed Forces temperament test gives information on genetic differences among dogs. J. Vet. Behav: Clin. Appl. Res. 9 (6), 281e289.

Asher, L., Blythe, S., Roberts, R., Toothill, L., Craigon, P.J., Evans, K.M., Green, M.J., England, G.C.W., 2013. A standardized behavior test for potential guide dog puppies: methods and association with subsequent success in guide dog training. J. Vet. Behav: Clin. Appl. Res. 8, 431e438.

Batt, L., Batt, M., Baguley, J., McGreevy, P., 2010. Relationships between puppy management practices and reported measures of success in guide dog training. J. Vet. Behav: Clin. Appl. Res. 5, 240e246.

Batt, L.S., Batt, M.S., Baguley, J.A., McGreevy, P.D., 2008. Factors associated with success in guide dog training. J. Vet. Behav: Clin. Appl. Res. 3, 143e151.

Battaglia, C.L., 2009. Periods of early development and the effects of stimulation and social experiences in the canine. J. Vet. Behav: Clin. Appl. Res. 4, 203e210.

Benetti, F., de Araujo, P.A., Sanvitto, G.L., Lucion, A.B., 2007. Effects of neonatal novelty exposure on sexual behavior, fear, and stress-response in adult rats. Dev. Psychobiol. 49, 258e264.

Bennett, P.C., Rohlf, V.I., 2007. Owner-companion dog interactions: relationships between demographic variables, potentially problematic behaviours, training engagement and shared activities. Appl. Anim. Behav. Sci. 102, 65e84.

Bijlsma, E.Y., de Jongh, R., Olivier, B., Groenink, L., 2010. Fear-potentiated startle, but not light-enhanced startle, is enhanced by anxiogenic drugs. Pharm. Biochem. Behav. 96, 24e31.

Blackwell, E.J., Bodnariu, A., Tyson, J., Bradshaw, J.W.S., Casey, R.A., 2010. Rapid shaping of behaviour associated with high urinary cortisol in domestic dogs. Appl. Anim. Behav. Sci. 124, 113e120.
Blackwell, E., Bradshaw, J., Casey, R., 2013. Fear responses to noises in domestic dogs: prevalence, risk factors and co-occurrence with other fear related behaviour. Appl. Anim. Behav. Sci. 145, 15e25.
Blackwell, E.J., Twells, C., Seawright, A., Casey, R.A., 2008. The relationship between training methods and the occurrence of behavior problems, as reported by owners, in a population of domestic dogs. J. Vet. Behav: Clin. Appl. Res. 3, 207e217.
Boissy, A., 1998. Fear and fearfulness in determining behavior. In: Grandin, T. (Ed.), Genetics and the Behavior of Domestic Animals. Academic Press, New York, pp. 67e111.
Bosch, O.J., Müsch, W., Bredewold, R., Slattery, D.A., Neumann, I.D., 2007. Prenatal stress increases HPA axis activity and impairs maternal care in lactating female offspring: implications for postpartum mood disorder. Psychoneuroendocrinology 32, 267e278.
Boxall, J., Heath, S., Bate, S., Brautigam, J., 2004. Modern concepts of socialization for dogs: implications for their behaviour, welfare and use in scientific procedures. Altern. Lab. Anim. 32, 81e93.
Bradshaw, J.W.S., Blackwell, E.J., Casey, R.A., 2009. Dominance in domestic dogsuseful construct or bad habit? J. Vet. Behav: Clin. Appl. Res. 4, 135e144.
Branson, N.J., Rogers, L.J., 2006. Relationship between paw preference strength and noise phobia in Canis familiaris. J. Comp. Psychol. 120, 176e183.
Brown, J., 2011. Developing the Disaster Search Dog, Defining, Developing and Documenting Success in Working Dogs. Pearl River, New York.
Burghardt, W.F., 2003. Behavioral considerations in the management of working dogs. Vet. Clin. North Am. Small Anim. Pract. 33, 417e446.
Caron-Lormier, G., Harvey, N.D., England, G.C., Asher, L., 2016. Using the incidence and impact of behavioural conditions in guide dogs to investigate patterns in undesirable behaviour in dogs. Sci. Rep. 6, 23860.
Casey, R., 2013. Should Fearful Dogs Be Comforted on Fireworks Night? Reigning Cats and Dogs: A Scientific Perspective on Companion Animal Behaviour and Welfare, by Veterinary Behaviourist Dr Rachel Casey. Available at: https:// behaviourvet.wordpress.com/2013/11/04/should-fearful-dogs-be-comfortedon-fireworks-night/. Accessed August 16, 2015.
Casey, R., 2010. In: Lindley, S., Watson, P. (Eds.), BSAVA Manual of Canine and Feline Rehabilitation, Supportive and Palliative Care: Case Studies in Patient Management. British Small Animal Veterinary Association, Gloucester, UK.
Casey, R.A., Bradshaw, J.W.S., 2008. The effects of additional socialization for kittens in a rescue centre on their behaviour and suitability as a pet. Appl. Anim. Behav. Sci. 114, 196e205.
Casey, R., Loftus, B., Bolster, C., Richards, G., Blackwell, E., 2014. Human directed aggression in domestic dogs (Canis familiaris): occurrence in different contexts and risk factors. Appl. Animal Behav. Sci. 152, 52e63.
Champness, K.A., 1996. Development of a Breeding Program for Drug Detector DogsdBased on Studies of a Breeding Population of Guide Dogs, PhD Thesis University of Melbourne. Available at: https://minerva-access.unimelb.edu.au/ handle/11343/39395.
Christensen, J.W., Rundgren, M., Olsson, K., 2006. Training methods for horses: habituation to a frightening stimulus. Equine Vet. J. 38, 439e443.
Cobb, M., Branson, N., McGreevy, P., Bennett, P., Rooney, N., Magdalinski, T., Howell, T., Dawson, K., 2015. Review & Assessment of Best Practice Rearing, Socialization, Education and Training Methods for Greyhounds in a Racing Context. Report to Greyhound Racing New South Wales. Available at: http:// workingdogalliance.com.au/about/publications/.
Concannon, P.W., Butler, W.R., Hansel, W., Knight, P.J., Hamilton, J.M., 1978. Parturition and lactation in the bitch: serum progesterone, cortisol and prolactin. Biol. Reprod. 19, 1113e1118.

Coppola, C.L., Grandin, T., Enns, R.M., 2006. Human interaction and cortisol: can human contact reduce stress for shelter dogs? Physiol. Behav. 87, 537e541.
D'Eath, R.B., Conington, J., Lawrence, A.B., Olsson, I.A.S., Sandoe, P., 2010. Breeding for behavioural change in farm animals: practical, economic and ethical considerations. Anim. Welf. 19, 17e27.
Dale, A.R., Walker, J.K., Farnworth, M.J., Morrissey, S.V., Waran, N.K., 2010. A survey of owners' perceptions of fear of fireworks in a sample of cats and dogs in New Zealand. N. Z. Vet. J. 58, 286e291.
Davis, M., 1974. Sensitization of rat startle response by noise. J. Comp. Physiol. Psychol. 87, 571e581.
de Boer, S.F., van der Vegt, B.J., Koolhaas, J.M., 2003. Individual variation in aggression of feral rodent strains: a standard for the genetics of aggression and violence? Behav. Genet. 33, 485.
De Meester, R.H., Pluijmakers, J., Vermeire, S., Laevens, H., 2011. The use of the socially acceptable behavior test in the study of temperament of dogs. J. Vet. Behav: Clin. Appl. Res. 6, 211e224.
Dodic, M., Hantzis, V., Duncan, J., Rees, S., Koukoulas, I., Johnson, K., Wintour, E.M., Moritz, K., 2002. Programming effects of short prenatal exposure to cortisol. FASEB J. 16, 1017e1026.
Dreschel, N.A., 2010. The effects of fear and anxiety on health and lifespan in pet dogs. Appl. Anim. Behav. Sci. 125, 157e162.
Dreschel, N.A., Granger, D.A., 2005. Physiological and behavioral reactivity to stress in thunderstorm-phobic dogs and their caregivers. Appl. Anim. Behav. Sci. 95, 153e168.
Ennaceur, A., Michalikova, S., Chazot, P.L., 2006. Models of anxiety: responses of rats to novelty in an open space and an enclosed space. Behav. Brain Res.171, 26e49.
Fabricius, K., Wörtwein, G., Pakkenberg, B., 2008. The impact of maternal separation on adult mouse behaviour and on the total neuron number in the mouse hippocampus. Brain Struct. Funct. 212, 403e441.
Fisher, G.T., Volhard, W., 1985. Puppy Personality Profile: The Specifics of Testing Procedures, Scoring, and Interpretation of the Results of PAT, Purebred Dog American Kennel Club Gazette. The Kennel Club Publisher, London, pp. 36e42.
Forkman, B., Furuhaug, I.L., Jensen, P., 1995. Personality, coping patterns and aggression in piglets. Appl. Anim. Behav. Sci. 45, 31e42.
Foyer, P., Svedberg, A.M., Nilsson, E., Wilsson, E., Faresjö, Å., Jensen, P., 2016. Behavior and Cortisol Responses of Dogs Evaluated in a Standardized Temperament Test for Military Working Dogs: 4th Canine Science Forum and 1st Feline Science Forum Lincoln. UK Journal of Veterinary Behvaior-Clinical Applications and Research, 11, 7e12.
Fratkin, J.L., Sinn, D.L., Patall, E.A., Gosling, S.D., 2013. Personality consistency in dogs: a meta-analysis. PLos One 8, e54907.
Gaines, S., 2008. Kennelled Dog WelfaredEffects of Housing and Husbandry, PhD Thesis University of Bristol. Available at: http://research-information. bristol.ac.uk/files/34503545/492636.pdf.
Gaines, S.A., Rooney, N.J., Bradshaw, J.W.S., 2008. The effect of feeding enrichment upon reported working ability and behavior of kenneled working dogs. J. Forensic Sci. 53, 1400e1404.
Gazzano, A., Mariti, C., Notari, L., Sighieri, C., McBride, E.A., 2008. Effects of early gentling and early environment on emotional development of puppies. Appl. Anim. Behav. Sci. 110, 294e304.
Goddard, M.E., Beilharz, R.G., 1986. Early prediction of adult behaviour in potential guide dogs. Appl. Anim. Behav. Sci. 15, 247e260.
Goddard, M.E., Beilharz, R.G., 1985. A multivariate analysis of the genetics of fearfulness in potential guide dogs. Behav. Genet. 15, 69e89.
Goddard, M.E., Beilharz, R.G., 1984. A factor analysis of fearfulness in potential guide dogs. Appl. Anim. Behav. Sci. 12, 253e265.
Goddard, M.E., Beilharz, R.G., 1984. The relationship of fearfulness to, and the effects of, sex, age and experience on exploration and activity in dogs. Appl. Anim. Behav. Sci. 12, 267e278.

Goddard, M.E., Beilharz, R.G., 1982. Genetic and environmental factors affecting the suitability of dogs as Guide Dogs for the Blind. Theor. Appl. Genet. 62, 97e102.
Gotz, T., Janik, V., 2011. Repeated elicitation of the acoustic startle reflex leads to sensitization in subsequent avoidance behaviour and induces fear conditioning. BMC Neurosci. 12, 30.
Grandin, T., Deesing, M., 2002. Distress in Animals: Is it Fear, Pain or Physical Stress? American Board of Veterinary Practitioners, Manhattan Beach, California, USA.
Grant, K.A., McMahon, C., Austin, M.P., Reilly, N., Leader, L., Ali, S., 2009. Maternal prenatal anxiety, postnatal caregiving and infants' cortisol responses to the stillface procedure. Dev. Psychobiol 51, 625e637.
Gray, J., 1971. The Psychology of Fear and Stress. McGraw Hill, New York.
Grissom, N., Bhatnagar, S., 2009. Habituation to repeated stress: get used to it. Neurobiol. Learn. Mem. 92, 215e224.
Gruen, M.E., Case, B.C., Foster, M.L., Lazarowski, L., Fish, R.E., Landsberg, G., Depuy, V., Dorman, D.C., Sherman, B.L., 2015. The use of an open-field model to assess sound-induced fear and anxiety-associated behaviors in Labrador retrievers. J. Vet. Behav: Clin. Appl. Res. 10, 338e345.
Grundy, S.A., Feldman, E., Davidson, A., 2002. Evaluation of infertility in the bitch. Clin. Tech. Small Anim. Pract. 17, 108e115.
Haverbeke, A., De Smet, A., Depiereux, E., Giffroy, J.M., Diederich, C., 2009. Assessing undesired aggression in military working dogs. Appl. Anim. Behav. Sci. 117, 55e62.
Haverbeke, A., Laporte, B., Depiereux, E., Giffroy, J.M., Diederich, C., 2008. Training methods of military dog handlers and their effects on the team's performances. Appl. Anim. Behav. Sci. 113, 110e122.
Haverbeke, A., Messaoudi, F., Depiereux, E., Stevens, M., Giffroy, J.M., Diederich, C., 2010. Efficiency of working dogs undergoing a new Human Familiarization and Training Program. J. Vet. Behav: Clin. Appl. Res. 5, 112e119.
Hayward, J.J., Castelhano, M.G., Oliveira, K.C., Corey, E., Balkman, C., Baxter, T.L., Casal, M.L., Center, S.A., Fang, M., Garrison, S.J., Kalla, S.E., Korniliev, P., Kotlikoff, M.I., Moise, N.S., Shannon, L.M., Simpson, K.W., Sutter, N.B., Todhunter, R.J., Boyko, A.R., 2016. Complex disease and phenotype mapping in the domestic dog. Nat. Commun. 7, 10460.
Heilig, M., 2004. The NPY system in stress, anxiety and depression. Neuropeptides 38, 213e224.
Hellemans, K.G.C., Sliwowska, J.H., Verma, P., Weinberg, J., 2010. Prenatal alcohol exposure: fetal programming and later life vulnerability to stress, depression and anxiety disorders. Neurosci. Biobehav. Rev. 34, 791e807.
Hennessy, M.B., Morris, A., Linden, F., 2006. Evaluation of the effects of a socialization program in a prison on behavior and pituitary-adrenal hormone levels of shelter dogs. Appl. Anim. Behav. Sci. 99, 157e171.
Hennessy, M.B., Williams, M.T., Miller, D.D., Douglas, C.W., Voith, V.L., 1998. Influence of male and female petters on plasma cortisol and behaviour: can human interaction reduce the stress of dogs in a public animal shelter? Appl. Anim. Behav. Sci. 61, 63e77.
Hiby, E.F., Rooney, N.J., Bradshaw, J.W.S., 2004. Dog training methods: their use, effectiveness and interaction with behaviour and welfare. Anim. Welf. 13, 63e69.
Hoffmann, G., Blackshaw, J.K., Smith, G.A., 1995. Puppy Tests: Intercorrelations and Test-retest Reliability, Proceedings of the 7th International Conference of Human-animal Interactions, Geneva.
Holden, M.D., Gregory, J., Watkins, V., Radford, L., 2006. Operant-conditioning Program for White Rhinoceros, Black Rhinoceros and Indian or Greater Onehorned Asian Rhinoceros Ceratotherium simum, Diceros bicornis and Rhinoceros Unicornis at Whipsnade Wild Animal Park. International Zoo Yearbook 40, Dunstable, UK, pp. 144e149.
Horvath, Z., Igyarto, B.Z., Magyar, A., Miklosi, A., 2007. Three different coping styles in police dogs exposed to a short-term challenge. Horm. Behav. 52, 621e630.

Houpt, K.A., Hintz, H.F., 1983. Some effects of maternal-deprivation on maintenance behavior, spatial relationships and responses to environmental novelty in foals. Appl. Anim. Ethol. 9, 221e230.
Howell, T.J., Bennett, P.C., 2011. Puppy power! Using social cognition research tasks to improve socialization practices for domestic dogs (Canis familiaris). J. Vet. Behav: Clin. Appl. Res. 6, 195e204.
Hsu, Y.Y., Serpell, J.A., 2003. Development and validation of a questionnaire for measuring behavior and temperament traits in pet dogs. J. Am. Vet. Med. Assoc. 223, 1293.
Hsu, Y.Y., Sun, L.C., 2010. Factors associated with aggressive responses in pet dogs. Appl. Animal Behav. Sci. 123, 108e123.
Imanaka, A., Morinobu, S., Toki, S., Yamamoto, S., Matsuki, A., Kozuru, T., Yamawaki, S., 2008. Neonatal tactile stimulation reverses the effect of neonatal isolation on open-field and anxiety-like behavior, and pain sensitivity in male and female adult Sprague-Dawley rats. Behav. Brain Res. 186, 91e97.
Jagoe, A., Serpell, J., 1996. Owner characteristics and interactions and the prevalence of canine behaviour problems. Appl. Anim. Behav. Sci. 47, 31e42.
Jarvis, S., Moinard, C., Robson, S.K., Baxter, E., Ormandy, E., Douglas, A.J., Seckl, J.R., Russell, J.A., Lawrence, A.B., 2006. Programming the offspring of the pig by prenatal social stress: neuroendocrine activity and behaviour. Horm. Behav. 49, 68e80.
Jensen, P., Rushen, J., Forkman, B., 1995. Behavioral strategies or just individual variation in behaviourda lack of evidence for active and passive piglets. Appl. Anim. Behav. Sci. 43, 135e139.
Jones, A.C., Gosling, S.D., 2005. Temperament and personality in dogs (Canis familiaris): a review and evaluation of past research. Appl. Anim. Behav. Sci. 95, 1e53.
Jones, A.C., Josephs, R.A., 2006. Interspecies hormonal interactions between man and the domestic dog (Canis familiaris). Horm. Behav. 50, 393e400.
Jordan, W.P., Strasser, H.C., 2000. Contextual control of long-term habituation in rats. J. Exp. Psychol. Anim. Behav. Process. 26, 323e339.
Karagiannis, C.I., Burman, O.H., Mills, D.S., 2015. Dogs with separation-related problems show a "less pessimistic" cognitive bias during treatment with fluoxetine (Reconcile (TM)) and a behaviour modification plan. BMC Vet. Res. 11, 1e10.
Kilgour, R., Foster, T.M., Temple, W., Matthews, L.R., Bremner, K.J., 1991. Operant technology applied to solving farm animal problems. An assessment. Appl. Anim. Behav. Sci. 30, 141e166.
Knuth, E.D., Etgen, A.M., 2007. Long-term behavioral consequences of brief, repeated neonatal isolation. Brain Res. 1128, 139e147.
Koolhaas, J.M., Korte, S.M., De Boer, S.F., Van Der Vegt, B.J., Van Reenen, C.G., Hopster, H., De Jong, I.C., Ruis, M.A.W., Blokhuis, H.J., 1999. Coping styles in animals: current status in behavior and stress-physiology. Neurosci. Biobehav. Rev. 23, 925e935.
Kutzler, M.A., 2012. Canine Semen Collection and Management of Male Infertility, Fifth Congreso Latinoamericano de Emergencias y Cuidados Intensivos, Mexico.
Lambás-Señas, L., Mnie-Filali, O., Certin, V., Faure, C., Lemoine, L., Zimmer, L., Haddjeri, H., 2009. Functional correlates for 5-HT(1A) receptors in maternally deprived rats displaying anxiety and depression-like behaviors Prog. Neuropsychopharmacol. Biol. Psychiatry 33, 262e268.
Landsberg, G.M., Hunthausen, W.L., Ackerman, L.J., 2012. Behavior Problems of the Dog and Cat. Saunders Ltd, Edinburgh.
Latham, N.R., Mason, G.J., 2008. Maternal deprivation and the development of stereotypic behaviour. Appl. Anim. Behav. Sci. 110, 84e108.
Lefebvre, D., Diederich, C., Delcourt, M., Giffroy, J.M., 2007. The quality of the relation between handler and military dogs influences efficiency and welfare of dogs. Appl. Anim. Behav. Sci. 104, 49e60.
Leiner, L., Fendt, M., 2011. Behavioural fear and heart rate responses of horses after exposure to novel objects: effects of habituation. Appl. Anim. Behav. Sci.131,104e109.

Levin, R., Nielsen, T.A., 2007. Disturbed dreaming, posttraumatic stress disorder, and affect distress: a review and neurocognitive model. Psychol. Bull. 133, 482e528.
Levine, S., 2000. Influence of psychological variables on the activity of the hypothalamic-pituitary-adrenal axis. Eur. J. Pharmacol. 405, 149e160.
Levine, E.D., Ramos, D., Mills, D.S., 2007. A prospective study of two self-help CD based desensitization and counter-conditioning programs with the use of Dog Appeasing Pheromone for the treatment of firework fears in dogs (Canis familiaris). Appl. Anim. Behav. Sci. 105, 311e329.
Lin, H.P., Lin, H.Y., Lin, W.L., Huang, A.C.W., 2011. Effects of stress, depression, and their interaction on heart rate, skin conductance, finger temperature, and respiratory rate: sympathetic-parasympathetic hypothesis of stress and depression. J. Clin. Psychol. 67, 1080e1091.
Lippmann, M., Bress, A., Nemeroff, C.B., Plotsky, P.M., Monteggia, L.M., 2007. Longterm behavioural and molecular alterations associated with maternal separation in rats. Eur. J. Neurosci. 25, 3091e3098.
Lissek, S., Powers, A.S., McClure, E.B., Phelps, E.A., Woldehawariat, G., Grillon, C., Pine, D.S., 2005. Classical fear conditioning in the anxiety disorders: a metaanalysis. Behav. Res. Ther. 43, 1391e1424.
Loftus, B.A., Rooney, N.J., Casey, R.A., 2012. Recognising Fear and Anxiety in Dogs. Available at: http://www.bris.ac.uk/vetscience/services/behaviour-clinic/dogbehaviouralsigns/. Accessed September 10, 2016.
Mackenzie, S.A., Oltenacu, E.A.B., Leighton, E., 1985. Heritability estimate for temperament scores in German Shepherd dogs and its genetic correlation with hip-displasia. Behav.Genet. 15, 475e482.
Macrì, S., Würbel, H., 2006. Developmental plasticity of HPA and fear responses in rats: a critical review of the maternal mediation hypothesis. Horm. Behav. 50, 667e680.
Macri, S., Wurbel, H., 2007. Effects of variation in postnatal maternal environment on maternal behaviour and fear and stress responses in rats. Anim. Behav. 73, 171e184.
Macri, S., Granstrem, O., Shumilina, M., dos Santos, F., Berry, A., Saso, L., Laviola, G., 2009. Resilience and vulnerability are dose-dependently related to neonatal stressors in mice. Horm. Behav. 56, 391e398.
Macri, S., Laviola, G., Leussis, M.P., Andersen, S.L., 2010. Abnormal behavioral and neurotrophic development in the younger sibling receiving less maternal care in a communal nursing paradigm in rats. Psychoneuroendocrinology 35, 392e402.
Macri, S., Zoratto, F., Laviola, G., 2011. Early-stress regulates resilience, vulnerability and experimental validity in laboratory rodents through mother-offspring hormonal transfer. Neurosci. Biobehav. Rev. 35, 1534e1543.
Malinowski, K., Hallquist, N.A., Helyar, L., Sherman, A.R., Scanes, C.G., 1990. Effect of separation protocols between mares and foals on plasma cortisol and cellmediated immune response. J. Equine Vet. Sci. 10, 363.
Manteuffel, G., Langbein, J., Puppe, B., 2009. From operant teaming to cognitive enrichment in farm animal housing: bases and applicability. Anim. Welf. 18, 87e95.
Mariti, C., Gazzano, A., Moore, J.L., Baragli, P., Chelli, L., Sighieri, C., 2012. Perception of dogs' stress by their owners. J. Vet. Behav: Clin. Appl. Res. 7, 213e219.
Marshall-Pescini, S., Barnard, S., Branson, N.J., Valsecchi, P., 2013. The effect of preferential paw usage on dogs' (Canis familiaris) performance in a manipulative problem-solving task. Behav. Process. 100, 40e43.
Masini, C.V., Day, H.E.W., Campeau, S., 2008. Long-term habituation to repeated loud noise is impaired by relatively short interstressor intervals in rats. Behav. Neurosci. 122, 210e223.
Massar, S.A.A., Mol, N.M., Kenemans, J.L., Baas, J.M.P., 2011. Attentional bias in highand low-anxious individuals: evidence for threat-induced effects on engagement and disengagement. Cogn. Emot. 25, 805e817.
McCall, C.A., Potter, G.D., Kreider, J.L., 1985. Locomotor, vocal and other behavioural responses to varying methods of weaning foals. Appl. Anim. Behav. Sci. 14, 27e35.

McCall, C.A., Potter, G.D., Kreider, J.L., Jenkins, W.L., 1987. Physiological responses in foals weaned by abrupt or gradual methods. J. Equine Vet. Sci. 7, 368e374.
Memon, M.A., 2007. Common causes of male dog infertility. Theriogenology 68, 322e328.
Mendl, M., Brooks, J., Basse, C., Burman, O., Paul, E., Blackwell, E., Casey, R., 2010. Dogs showing separation-related behaviour exhibit a ‘pessimistic’ cognitive bias. Curr. Biol. 20, R839eR840.
Meunier, L.D., 2006. Selection, acclimation, training, and preparation of dogs for the research setting. ILAR J. 47, 326e347.
Mills, D., 2005. Management of noise fears and phobias in pets. Practice 27, 248e255.
Mills, D., Karagiannis, C., Zulch, H., 2014. Stress-its effects on health and behavior: a guide for practitioners. Vet. Clin. North Am: Small Anim. Pract. 44, 525e541.
Mineka, S., Ohman, A., 2002. Born to fear: non-associative vs associative factors in the etiology of phobias. Behav. Res. Ther. 40, 173e184.
Morgan, C.A., Southwick, S., Hazlett, G., Rasmusson, A., Hoyt, G., Zimolo, Z., Charney, D., 2004. Relationships among plasma dehydroepiandrosterone sulfate and cortisol levels, symptoms of dissociation, and objective performance in humans exposed to acute stress. Arch. Gen. Psychiatry 61, 819e825.
Mornement, K.M., Coleman, G.J., Toukhsati, S.R., Bennett, P.C., 2015. Evaluation of the predictive validity of the Behavioural Assessment for Re-homing K9’s (BARK) protocol and owner satisfaction with adopted dogs. Appl. Anim. Behav. Sci. 167, 35e42.
Morrow, M., Ottobre, J., Ottobre, A., Neville, P., St-Pierre, N., Dreschel, N., Pate, J., 2015. Breed-dependent differences in the onset of fear-related avoidance behavior in puppies. J. Vet. Behav: Clin. Appl. Res. 10 (4), 286e294.
Murphree, O.D., Dykman, R.A., 1965. Litter patterns in the offspring of nervous and stable dogs. 1. Behavioral-tests. J. Nerv. Ment. Dis. 141, 321e332.
Murphy, J.A., 1995. Assessment of the temperament of potential guide dogs.
Anthrozoös 8, 224e228.
Nyhuis, T.J., Sasse, S.K., Masini, C.V., Day, H.E.W., Campeau, S., 2010. Lack of contextual modulation of habituated neuroendocrine responses to repeated audiogenic stress. Behav. Neurosci. 124, 810e820.
Odendaal, J.S.J., Meintjes, R.A., 2003. Neurophysiological correlates of affiliative behaviour between humans and dogs. Vet. J. 165, 296e301.
Ogata, N., Kikusui, T., Takeuchi, Y., Mori, Y., 2006. Objective measurement of fearassociated learning in dogs. J. Vet. Behav: Clin. Appl. Res. 1, 55e61.
Parker, K.J., Buckmaster, C.L., Sundlass, K., Schatzberg, A.F., Lyons, D.M., 2006. Maternal mediation, stress inoculation, and the development of neuroendocrine stress resistance in primates. Proc. Natl. Acad. Sci. U. S. A. 103, 3000e 3005.
Pfaffenberger, C.J., Scott, J.P., 1976. Early rearing and testing. In: Pfaffenberger, C.J., Scott, J.P., Fuller, J.L., Ginsburg, B.E., Bielfelt, S.W. (Eds.), Guide Dogs for the Blind: Their Selection, Development, and Training. Elsevier, Amsterdam, pp. 13e37.
Pich, E.M., Agnati, L.F., Zini, I., Marrama, P., Carani, C., 1993. Neuropeptide-Y produces anxiolytic effects in spontaneously hypertensive rats. Peptides 14, 909e912.
Pluijmakers, J.J.T.M., Appleby, D.L., Bradshaw, J.W.S., 2010. Exposure to video images between 3 and 5 weeks of age decreases neophobia in domestic dogs. Appl. Anim. Behav. Sci. 126, 51e58.
Podberscek, A.L., Gosling, S.D., 2000. Personality research on pets and their owners: conceptual issues and review. In: Podberscek, A.L., Paul, E.S., Serpell, J.A. (Eds.), Companion Animals and Us: Exploring the Relationships between People and Pets. Cambridge University Press, Cambridge, pp. 143e167.
Podberscek, A.L., Serpell, J.A., 1997. Aggressive behaviour in English cocker spaniels and the personality of their owners. Vet. Rec. 141, 73e76.

Poore, K.R., Boullin, J.P., Cleal, J.K., Newman, J.P., Noakes, D.E., Hanson, M.A., Green, L.R., 2010. Sex- and age-specific effects of nutrition in early gestation and early postnatal life on hypothalamo-pituitary-adrenal axis and sympathoadrenal function in adult sheep. J. Physiol. 588, 2219e2237.
Reefmann, N., Kaszas, F.B., Wechsler, B., Gygax, L., 2009. Physiological expression of emotional reactions in sheep. Physiol. Behav. 98, 235e241.
Reefmann, N., Wechsler, B., Gygax, L., 2009. Behavioural and physiological assessment of positive and negative emotion in sheep. Anim. Behav. 78, 651e659.
Rooney, N.J., 2012. Welfare of Racing GreyhoundsdPrioritisation of Issues. Poster Presented at Universities Federation for Animal Welfare Portsmouth UK.
Rooney, N.J., Almney, H.A., Bradshaw, J.W.S., 2004. Attributes of specialist search dogsda questionnaire survey of UK dog handlers and trainers. J. Forensic Sci. 49 (2), 300e306.
Rooney, N., Bradshaw, J.W.S., Almey, H.A., 2002. Specialist Search Dogs: An Interagency Review and Critique of Selection, Report to DERA.
Rooney, N., Bradshaw, J.W.S., Gaines, S., 2003. Investigation Into the Effects of Rearing Conditions on the Ability of Specialist Search Dogs, Report to Defence Science and Technology Laboratory, pp. 1e229.
Rooney, N.J., Cowan, S., 2011. Training methods and owner-dog interactions: links with dog behaviour and learning ability. Appl. Anim. Behav. Sci. 132, 169e177.
Rooney, N.J., Gaines, S.A., Bradshaw, J.W.S., 2007a. Behavioural and glucocorticoid responses of dogs (Canis familiaris) to kennelling: Investigating mitigation of stress by prior habituation. Physiol. Behav. 92, 847e854.
Rooney, N., Gaines, S., Hiby, E., 2009. A practitioner’s guide to working dog welfare. J. Vet. Behav: Clin. Appl. Res. 4, 127e134.
Rooney, N.J., Morant, S., Guest, C., 2013. Investigation into the value of trained glycaemia alert dogs to clients with type I diabetes. PLoS One 8 (8), e69921.
Rooney, N.J., Paul, E.S., Haig-Ferguson, A.J., Bradshaw, J.W.S., 2007b. Human Factors Affecting the Performance of Dog-handler Teams, Report to Defence Science and Technology Laboratory.
Roth, S., Cohen, L.J., 1986. Approach, avoidance, and coping with stress. Am. Psychol 41, 813e819.
Sankey, C., Richard-Yris, M.A., Henry, S., Fureix, C., Nassur, F., Hausberger, M., 2010. Reinforcement as a mediator of the perception of humans by horses (Equus caballus). Anim. Cogn. 13, 753e764.
Savastano, G., Hanson, A., McCann, C., 2003. The development of an operant conditioning training program for new world primates at the Bronx Zoo. J. Appl. Anim. Welf. Sci. 6, 247e261.
Schalke, E., Stichnoth, J., Ott, S., Jones-Baade, R., 2007. Clinical signs caused by the use of electric training collars on dogs in everyday life situations. Appl. Anim. Behav. Sci. 105, 369e380.
Schilder, M.B.H., van der Borg, J.A.M., 2004. Training dogs with help of the shock collar:
short and long term behavioural effects. Appl. Anim. Behav. Sci. 85, 319e334.
Scott, J.P., Bielfelt,S.W., 1976. Analysis of the puppy testing program. In:
Pfaffenberger, C.J., Scott, J.P., Fuller, J.L., Ginsburg, B.E., Bielfelt, S.W. (Eds.), Guide Dogs for the Blind: Their Selection, Development, and Training. Elsevier, Amsterdam, pp. 39e76.
Scott, J.P., Fuller, J.L., 1965. Genetics and the Social Behavior of the Dog. University of Chicago Press, Chicago.
Seksel, K., Mazurski, E.J., Taylor, A., 1999. Puppy socialization programs: short and long term behavioural effects. Appl. Anim. Behav. Sci. 62, 335e349.
Serpell, J.A., Hsu, Y.Y., 2001. Development and validation of a novel method for evaluating behavior and temperament in guide dogs. Appl. Anim. Behav. Sci. 72, 347e364.
Sherman, B.L., Gruen, M.E., Case, B.C., Foster, M.L., Fish, R.E., Lazarowski, L., DePuy, V., Dorman, D.C., 2015. A test for the evaluation of emotional reactivity in Labrador retrievers used for explosives detection. J. Vet. Behav: Clin. Appl. Res. 10, 94e 102.

Sherman, B.L., Mills, D.S., 2008. Canine anxieties and phobias: an update on separation anxiety and noise aversions. Vet. Clin. North Am: Small Anim. Pract. 38, 1081e1106.
Shors, T.J., 2004. Learning during stressful times. Learn. Mem. 11, 137e144.
Silver, R.C., Holman, E.A., McIntosh, D.N., Poulin, M., Gil-Rivas, V., 2002. Nationwide longitudinal study of psychological responses to September 11. J. Am. Med. Assoc. 288, 1235e1244.
Siniscalchi, M., Bertino, D., Quaranta, A., 2014. Laterality and performance of agilitytrained dogs. Laterality 19, 219e234.
Sinn, D.L., Gosling, S.D., Hilliard, S., 2010. Personality and performance in military working dogs: reliability and predictive validity of behavioral tests. Appl. Anim. Behav. Sci. 127, 51e65.
Slabbert, J.M., Odendaal, J.S.J., 1999. Early prediction of adult police dog efficiencyda longitudinal study. Appl. Anim. Behav. Sci. 64, 269e288.
Slabbert, J.M., Rasa, O.A.E., 1997. Observational learning of an acquired maternal behaviour pattern by working dog pups: an alternative training method? Appl. Anim. Behav. Sci. 53, 309e316.
Slabbert, J.M., Rasa, O.A.E., 1993. The effect of early separation from the mother on pups in bonding to humans and pup health. J. S. Afr. Vet. Assoc. 64, 4e8.
Strong, V., Brown, S.W., Walker, R., 1999. Seizure-alert dogsdfact or fiction? Seizure 8, 62e65.
Svartberg, K., Forkman, B., 2002. Personality traits in the domestic dog (Canis familiaris). Appl. Anim. Behav. Sci. 79, 133e155.
Svobodová, I., Vápeník, P., Pinc, L., Bartos, L., 2008. Testing german shepherd puppies to assess their chances of certification. Appl. Anim. Behav. Sci. 113, 139e149.
Tami, G., Gallagher, A., 2009. Description of the behaviour of domestic dog (Canis familiaris) by experienced and inexperienced people. Appl. Anim. Behav. Sci. 120, 159e169.
Tata, D.A., Markostamou, I., Ioannidis, A., Gkioka, M., Simeonidou, C., Anogianakis, G., Spandou, E., 2015. Effects of maternal separation on behavior and brain damage in adult rats exposed to neonatal hypoxia-ischemia. Behav. Brain Res. 280, 51e61.
Taylor, K.D., Mills, D.S., 2006. The development and assessment of temperament tests for adult companion dogs. J. Vet. Behav: Clin. Appl. Res. 1, 94e108.
Terlouw, E.M.C., Schouton, W.G.P., Ladewig, P., 1997. Physiology. In: Appleby, M.C., Hughes, B.O. (Eds.), Anim. Welf. CAB International, Wallingford.
Thomas, S., 2011. Development and Epigeneticsdthe New Nature-nurture Discussion, Defining, Developing and Documenting Success in Working Dogs. Pearl River, New York.
Trimmer, P.C., Houston, A.I., Marshall, J.A.R., Bogacz, R., Paul, E.S., Mendl, M.T., McNamara, J.M., 2008. Mammalian choices: combining fast-but-inaccurate and slow-but-accurate decision-making systems. Proc. Biol. Sci. 275, 2353e2361.
Tuber, D.S., Sanders, S., Hennessy, M.B., Miller, J.A., 1996. Behavioral and glucocorticoid responses of adult domestic dogs (Canis familiaris) to companionship and social separation. J. Comp. Psychol. 110, 103e108.
Valentiner, D.P., Holahan, C.J., Moos, R.H., 1994. Social support, appraisals of event controllability, and coping: An integrative model. J. Pers. Soc. Psychol 66, 1094e1102.
Vincent, I.C., Leahy, R.A., 1997. Real-time non-invasive measurement of heart rate in working dogs: a technique with potential applications in the objective assessment of welfare problems. Vet. J. 153, 179e183.
Walker, J., Waran, N., Phillips, C., 2014. The effect of conspecific removal on the behaviour and physiology of pair-housed shelter dogs. Appl. Anim. Behav. Sci. 158, 46e56.
Wells, D.L., Hepper, P.G., 2006. Prenatal olfactory learning in the domestic dog. Anim. Behav. 72, 681e686.
Willis, M.B., 1995. Genetic aspects of dog behaviour with particular reference to working ability. In: Serpell, J. (Ed.), The Domestic Dog: Its Evolution, Behaviour, and Interactions with People. Cambridge University Press, Cambridge, pp. 51e64.
Wilson, D.S., Clark, A.B., Coleman, K., Dearstyne, T., 1994. Shyness and boldness in humans and other animals. Trends Ecol. Evol. 9, 442e446.

Wilsson, E., 2016. Nature and nurture-how different conditions affect the behavior of dogs. J. Vet. Behav.: Clin. Appl. Res. this volume.
Wilsson, E., Sinn, D.L., 2012. Are there differences between behavioral measurement methods? A comparison of predictive validity of two rating methods in a working dog program. Appl. Anim. Behav. Sci. 141 (3-4), 158e172.
Wilsson, E., Sundgren, P.E., 1997. The use of a behaviour test for the selection of dogs for service and breeding, I: Method of testing and evaluating test results in the adult dog, demands on different kinds of service dogs, sex and breed differences. Appl. Anim. Behav. Sci. 53, 279e295.
Winslow, J.T., Parr, L.A., Davis, M., 2002. Acoustic startle, prepulse inhibition, and fear-potentiated startle measured in rhesus monkeys. Biol. Psychiatry 51, 859e 866.
Yehuda, R., Brand, S., Yang, R.K., 2006. Plasma neuropeptide y concentrations in combat exposed veterans: relationship to trauma exposure, recovery from PTSD, and coping. Biol. Psychiatry 59, 660e663.
Yehuda, R., Flory, J.D., Southwick, S., Charney, D.S., 2006. Developing an Agenda for Translational Studies of Resilience and Vulnerability Following Trauma Exposure. Annals of the New York Academy of Sciences 1071 (Psychobiology of Posttraumatic Stress Disorder: A Decade of Progress), Wiley, pp. 379e396.